observed decreased $p16^{INK4a}$. Detailed analysis showed that HLX1 affects *INK4a* expression at the mRNA level and HLX1 binds to the *INK4a* promoter as observed by ChIP. We also observed increased levels of the repressive H3K27me3 mark and recruitment of PRC2 components at the *Ink4a*/*Arf* locus correlating with HLX1 expression. Co-immunoprecipitation studies showed that HLX1 associates with the PRC2, in particular with Suz12. RNAi studies showed that the repression of $p16^{Ink4a}$ by HLX1 is dependent of PRCs. In an attempt to understand if the repression of HLX1 was a property shared by other homeobox genes, we tested 20 homeobox-containing genes and identified that multiple homeobox can also repress $p16^{INK4a}$.

Conclusions: We identified the homeobox protein HLX1 as a novel $p16^{INK4a}$ repressor. HLX1 binds to the *INK4a* promoter region and recruits Polycomb repressive complexes. Multiple homeobox proteins can also regulate $p16^{INK4a}$ expression, which implies a conserved role for this family oftranscription factors in regulating the *Ink4a*/*Arf* locus, highlighting its potential physiological relevance for both senescence and carcinogenesis.

1004 ORAL

Lactate Influx and Efflux Through Monocarboxylate Transporters Bridge Cancer Cell Metabolism and Angiogenesis

F. Vegran[1], R. Boidot[1], P. Sonveaux[1], O. Feron[1]. *[1]UCL Institute of Experimental and Clinical Research (IREC), Angiogenesis and Cancer Research Lab, Brussels, Belgium*

Background: Tumour cells fuel their metabolism with a variety of nutrients to meet the bioenergetic and biosynthetic demands of proliferation. In particular, conversion of pyruvate into lactate allows the production of intracellular NAD^+ to maintain high glycolytic flux in tumours. Here, we investigated whether the consecutive lactate accumulation in the tumour microenvironment could indirectly modulate the endothelial cell phenotype and thereby promote angiogenesis.

Materials and Methods: Microfluidic low-density arrays were used to examine the influence of lactate on the endothelial expression profile of angiogenesis-related genes. The consecutive identification of IL-8/CXCL8 mRNA as the major upregulated transcript in response to lactate led us to study the signaling pathway bridging lactate and IL-8-driven angiogenesis using dedicated gene silencing and pharmacological strategies. We also addressed the *in vivo* relevance of this pathway in different mouse tumour models combining the injection of shRNA-transduced tumour and endothelial cells into extracellular matrix plugs.

Results: We found that lactate could enter endothelial cells through the monocarboxylate transporter MCT-1 and then stimulate an autocrine NFkB/IL-8 (CXCL8) pathway driving endothelial cell migration and tube formation. We further identified the capacity of lactate to activate NFkB through the phosphorylation and consecutive degradation of IkBa. These effects were prevented by 2-oxoglutarate and reactive oxygen species (ROS) inhibitors, pointing to a role for prolyl-hydroxylase and ROS in the integration of lactate signaling in endothelial cells. Prolyl-hydroxylase PHD2 silencing in glucose-fuelled endothelial cells recapitulated the pro-angiogenic effects of lactate, whereas a blocking IL-8 antibody or IL-8-targeting siRNA prevented them. Finally, we documented in mouse xenograft models of human colorectal and breast cancers that lactate release from tumour cells through the MCT4 (and not MCT1) transporter was sufficient to stimulate IL-8-dependent angiogenesis and tumour growth.

Conclusions: Our findings establish the existence of a lactate-driven feed-forward IL-8 autocrine loop driving angiogenesis in tumours and the key roles of monocarboxylate transporters MCT1 and MCT4 in this lactate-based dialog between cancer cells and endothelial cells. More generally, our study provides a new rationale for associating elevated lactate concentrations in tumours and negative outcomes for patients, and further supports the current enthusiasm for new cancer treatments targeting metabolic pathways.

1005 ORAL

HER-3, IGF-1, NF K-B and EGFR Gene Copy Number in the Prediction of Clinical Outcome for Colorectal Cancer Patients Receiving Irinotecan-cetuximab

M. Scartozzi[1], R. Giampieri[1], E. Maccaroni[1], A. Mandolesi[2], A. Zaniboni[3], L. Fotios[4], R. Berardi[1], A. Falcone[4], I. Bearzi[2], S. Cascinu[1]. *[1]AO Ospedali Riuniti, Oncologia Medica, Ancona, [2]AO Ospedali Riuniti, Anatomia Patologica, Ancona, [3]Casa di Cura Poliambulanza, Oncologia Medica, Brescia, [4]AO Pisa, Oncologia Medica, Pisa, Italy*

Background: A large proportion of colorectal cancer patients does not benefit from the use of anti-EGFR treatment although in the absence of a mutation of the K-RAS gene. Preliminary observations suggested that HER-3, IGF-1, NF-kB and EGFR GCN might identify patients not likely to benefit from anti-EGFR therapy. We tested the interaction between HER-3, IGF-1, NF-KB, EGFR GCN and K-RAS mutational analysis to verify the relative ability of these variables to identify a sub-group of patients more likely to benefit from EGFR-targeted treatment among those harbouring a K-RAS wild type status.

Materials and Methods: We retrospectively collected tumours from 168 patients with metastatic colorectal cancer patients treated with irinotecan-cetuximab. KRAS was assessed with direct sequencing, EGFR amplification was assessed by chromogenic in situ hybridization and HER-3, IGF-1 and NF-kB were assessed by immunoistochemistry.

Results: In patients with K-RAS wild type tumours, the following molecular factors resulted independently associated with response rate: HER-3 (OR = 4.6, 95% CI: 1.8–13.6, p = 0.02), IGF-1 (OR = 4.2, 95% CI: 2–10.2, p = 0.003) and EGFR GCN (OR = 4.1, 95% CI: 1.9–26.2, p = 0.04). These factors also independently correlated with overall survival as follows: HER-3 (HR = 0.4, 95% CI: 0.28–0.85, p = 0.008), IGF-1 (HR = 0.47, 95% CI: 0.24–0.76, p < 0.0001) and EGFR GCN (HR = 0.59, 95% CI: 0.22–0.89, p = 0.04) (table 1).

Conclusions: We believe that our data may help further composing the molecular mosaic of EGFR resistant tumours. HER-3, IGF-1 and CISH EGFR GCN proved to possess a relevant role in defining subgroups of colorectal cancer patients more likely to benefit from anti-EGFR treatment. Interestingly HER-3 and the IGF-1 driven pathway have also been demonstrated to be possible molecular targets as part of a treatment protocol focused on control of either HER receptors or the PI3K/AKT pathway. The possibility to use HER-3 and IGF-1 inhibitors in biologically-selected anti-EGFR resistant tumours promise then to be a crucial challenge for the future development of targeted therapy in colorectal cancer patients.

Table 1

	HER-3		IGF-1		EGFR	
	Positive (n = 46)	Negative (n = 44)	Positive (n = 59)	Negative (n = 31)	<2.12 (n = 47)	⩾2.12 (n = 43)
Response Rate (%)	25%	50%	22%	65%	6%	37%
Multivariate OR (95% CI	4.6 (1.8–13.6)		4.2 (2–10.2)		4.1 (1.9–26.2)	
Logistic regression *p* value	0.02		0.003		0.04	
Median Overall Survival (months)	11.3	25	8.3	25	10.4	18
Multivariate HR (95% CI)	0.4 (0.28–0.85)		0.47 (0.24–0.76)		0.59 (0.22–0.89)	
Cox regression *p* value	0.008		<0.0001		0.04	

1006 ORAL

Prognostic Factors for Progression-free Survival (PFS), Overall Survival (OS), and Long-term OS (LT-OS) With Sunitinib in 1,059 Patients, Treated on Clinical Trials, With Metastatic Renal Cell Carcinoma (mRCC)

R.J. Motzer[1], B. Escudier[2], R. Bukowski[3], B.I. Rini[3], T.E. Hutson[4], C.H. Barrios[5], X. Lin[6], K. Fly[7], E. Matczak[8], M.E. Gore[9]. *[1]Memorial Sloan Kettering Cancer Center, Department of Medicine, New York, USA; [2]Institut Gustave Roussy, Immunotherapy Unit, Villejuif, France; [3]Cleveland Clinic Taussig Cancer Institute, Solid Tumour Oncology, Cleveland, [4]Baylor Sammons Cancer Center-Texas Oncology, Internal Medicine, Dallas, USA; [5]PUCRS School of Medicine, Hematology and Oncology, Porto Alegre, Brazil; [6]Pfizer Oncology, Clinical Statistics, La Jolla, [7]Pfizer Oncology, Oncology, New London, [8]Pfizer Oncology, Oncology, New York, USA; [9]Royal Marsden Hospital NHS Trust, Medicine, London, United Kingdom*

Background: With the advent of multiple targeted therapies for mRCC, further information on factors affecting prognosis facilitates both clinical decision making and trial design for evaluation of new therapies. Here, we report a retrospective analysis of prognostic factors for PFS, OS and LT-OS (⩾30 months) in patients (pts) with mRCC treated with sunitinib in 6 clinical trials (NCT00054886, NCT00077974, NCT00083889, NCT00338884, NCT00137423, NCT00267748; Pfizer).

Methods: Analyses used pooled data from 1,059 pts treated with single-agent sunitinib on the approved 50 mg/day 4-week-on/2-week-off schedule (n = 689; 65%) or 37.5 mg continuous once-daily dosing (n = 370; 35%), in the first- (n = 783; 74%) or second-line (n = 276; 26%) setting. Baseline variables were analyzed for prognostic significance using a Cox proportional hazards model, with each factor investigated in univariate and then multivariate analyses using a stepwise algorithm.

Results: Multivariate analysis of PFS and OS identified 9 and 10 independent predictors, respectively (Table). Overall, 215 pts (20%) survived at least 30 months. An analysis of baseline characteristics of these long-term survivors showed characteristics differed between these pts and non-long-term survivors, including risk status based on the published Memorial Sloan-Kettering Cancer Center (MSKCC) prognostic criteria (Motzer, 2002; P < 0.0001). For example, 70% of the long-term survivors had favorable risk features compared with 31% of non-long-term

survivors. In contrast, 42% and 5% of the non-long-term survivors had intermediate and poor risk features compared with 28% and 0% of long-term survivors, respectively. Additional characteristics associated with LT-OS will be presented.

Variable	PFS		OS	
	HR (95% CI)	P-value*	HR (95% CI)	P-value*
Ethnic origin (white vs non-white)	0.598 (0.459, 0.781)	0.0002	0.730 (0.535, 0.996)	0.0474
ECOG PS† (⩾1 vs 0)	1.250 (1.043, 1.498)	0.0159	1.505 (1.218, 1.859)	0.0002
Time from diagnosis to treatment† (⩾1 vs <1 year)	0.814 (0.680, 0.975)	0.0252	0.666 (0.541, 0.820)	0.0001
Bone metastases (yes vs no)	–	–	1.535 (1.250, 1.886)	<0.0001
Baseline hemoglobin† (⩽LLN vs >LLN)	1.384 (1.144, 1.675)	0.0008	1.548 (1.245, 1.925)	<0.0001
Baseline lactate dehydrogenase† (>1.5 × ULN vs ⩽1.5 × ULN)	1.664 (1.201, 2.305)	0.0022	1.571 (1.103, 2.238)	0.0123
Baseline corrected calcium† (>10 vs ⩽10 mg/dL)	1.374 (1.080, 1.747)	0.0096	2.208 (1.722, 2.832)	<0.0001
Baseline neutrophils (⩽ULN vs >ULN)	0.629 (0.483, 0.821)	0.0006	0.681 (0.508, 0.915)	0.0107
Baseline platelets (⩽ULN vs >ULN)	0.607 (0.469, 0.785)	0.0001	0.670 (0.505, 0.889)	0.0055
Prior cytokine (yes vs no)	1.342 (1.085, 1.659)	0.0066	1.387 (1.094, 1.759)	0.0068

*Wald Chi-Square Test; †Factor included in MSKCC prognostic model

Conclusions: These analyses validated use of clinical risk factors previously reported from MSKCC (J Clin Oncol 20: 286, 2002) and by Heng et al (J Clin Oncol 27: 5794, 2009). These factors were predictive for shorter PFS as well. In addition, pts with bone metastases had shorter OS to sunitinib. Favorable MSKCC risk status was associated with higher likelihood of achieving LT-OS. Continued progress requires incorporation of RCC tumour-specific biology.

1007 ORAL

Efficacy of DNA Vaccination Against Anaplastic Lymphoma Kinase (ALK) in Non Small Cell Lung Carcinoma (NSCLC)

C. Voena[1], C. Mastini[1], M. Menotti[1], F. Di Giacomo[1], D.L. Longo[2], C. Martinengo[1], S. Aime[2], G. Inghirami[1], R. Chiarle[1]. [1]*Università Degli Studi, Department of Biomedical Sciences and Human Oncology, Torino,* [2]*Università Degli Studi, Department of Chemistry IFM, Torino, Italy*

Background: Lung cancer is the leading cause of cancer-related mortality worldwide. Recently, NSCLC harbouring ALK translocations have been described. Although standard chemotherapy or molecularly targeted therapies are effective in NSCLC, tumour recurrence and metastatic dissemination still remain a frequent event. Our previous findings show that ALK is an effective oncoantigen for ALK positive lymphoma vaccination and thus it could, as well, represent a feasible target for ALK positive NSCLC therapy.
Materials and Methods: We generated transgenic (Tg) mice ectopically expressing human TFG- or EML4-ALK protein in lung epithelium under the murine lung specific SP-C promoter. For DNA vaccination, we injected 50 ug of plasmid DNA in the femoral muscle of anesthetized mice for a total of at least 3 immunization, as previously described (*Chiarle et al., Nature Medicine 2008*). To evaluate the generation of an immune response, we performed an *in vivo* citotoxicity assay with CSFE-labelled cells one month after vaccination. Histology and immunohistochemistry were performed on different specimens. Tumour growth and progression was monitored overtime by Nuclear Magnetic Resonance (NMR).
Results: ALK Tg mice developed multifocal adenocarcinomas similar to human tumours, starting from 1 month after birth. A strong ALK specific CTL response was elicited in ALK positive vaccinated mice, thus demonstrating that ALK vaccination could overcome the immune tolerance to the ALK protein. By MRI analysis, vaccinated mice showed a reduced number of neoplastic foci and a smaller tumour mass as compared to mice vaccinated with a mock plasmid. The efficacy of DNA vaccination was dependent on mice age as the specific CTL activity against ALK and the ability to limit the tumour expansion decreased proportionally to the mice age. The number of T lymphocytes infiltrating both the tumours and the spared lung was significantly increased in vaccinated mice.
Conclusions: Our Tg mice represent a suitable model to dissect the role of ALK in lung tumour pathogenesis and for the development of innovative treatment strategies. Our findings indicate that ALK-DNA vaccination is able to elicit a specific cytotoxic response and to delay tumour progression in ALK+ Tg mice. Therefore, in ALK positive NSCLC a strategy that combines DNA vaccination combined with standard chemotherapy or specific ALK inhibitors could represent an alternative treatment to prevent tumour relapse or metastasis.

Poster Presentations (Sat, 24 Sep, 14:00–16:30)
Basic Science

1008 POSTER

The Effects of Telomerase Inhibitor GRN163L(Imetelstat) on Cell Cytoskeleton, Cell Cycle and Matrix Metalloproteinases

I. Mender[1], Z. Dikmen[1], S. Gryaznov[2], D. Kletsas[3], K. Erbil[4]. [1]*Hacettepe University, Clinical Biochemistry, Ankara, Turkey;* [2]*Geron Corporation, Clinical Biochemistry, California, USA;* [3]*Biology, Cell Proliferation and Ageing, Athens, Greece;* [4]*Gulhane School of Medicine, Clinical Chemistry, Ankara, Turkey*

Background: As telomerase activity can not be determined in somatic tissues but can be determined in 90% of human tumours, it is an attractive target for cancer therapy to telomerase. GRN163L is an N3′ → P5′-*thio-phosphoramidate* oligonucleotite which is complementary to the template region of telomerase RNA. We have previously reported that A549-luc cells treated before cell attachment with a single dose of GRN163L weakly attached to the substrate and remained rounded, whereas control cells exhibited typical epitheloid appearance and adhesion properties. In this study, we aimed to determine whether cell cytockeleton and adhesion proteins are relative with rapid morphologic alterations and loss of adhesion in GRN163L treated A549 cells. In addition, we investigated the potential decrease in MMP levels in GRN163L treated cells and also performed cell cycle analyses.
Material and Methods: A549 cells were plated in the presence of GRN163L (1 mM) and incubated for 24hrs. The untreated control cells and treated cells were collected following 24 hr of GRN163L incubation, then actin, tubulin and e-cadherin expressions were analysed by both Western Blot and immunohistochemistry. Real-Time PCR assay was used for cell cycle analyses and determination of MMP mRNA expressions of A549 lung cancer cells treated with GRN163L.
Results: We observed that actin, tubulin and e-cadherin expressions of GRN163L treated cells were significantly decreased within 24 hrs compared with the untreated control cells. Immunohistochemistry results also showed that all the actin and tubulin filaments were displaced and concentrated along the cell membrane. Interestingly, all of the effects were reversible after 72 hrs due to the the cessation of treatment. Additionally, according to Real-time PCR results, it was obvious that Cdk 6, cdk 4 and cyclin D1 mRNA levels that regulate the G1 phase of the cell cycle decreased following 1 week of GRN163L treatment when compared with the controls. Besides these results, MMP-2 expression of A549 cancer cells decreased following 24hrs of GRN163L treatment, but there was no significant change in MMP1 expression.
Conclusions: We can conclude that the morphological changes in cell cytoskeleton and loss of adhesion occur which ocur within 24 hr in GRN163L treated cells. These target-off effects besides telomerase inhibition decrease the adhesion, proliferation and metastatic potential of A549 cancer cells. For this reason, it may be possible to inhibit metastasis of residuel cancer cells by combining GRN163L with other chemotherapeutics or surgery.

1009 POSTER

Mammary Gland Tumour Formation in Conditional Transgenic Mice Expressing GLI1

J.H. Norum[1], M. Kasper[1], V. Jaks[1], R. Toftgård[1]. [1]*Karolinska Institutet, Department of Biosciences and Nutrition, Stockholm, Sweden*

Background: Up regulation of the Hedgehog pathway effector GLI1 in breast cancer correlates with unfavourable overall survival. The Hedgehog pathway has a role in the regulation and maintenance of CD44 positive breast cancer stem cells. Skin and intestinal stem cells express the orphan G protein coupled receptor (GPCR) LGR5. Previously, we have shown that multiparous conditional transgenic mice (MMTVrtTA;TREGLI1) expressing GLI1 develop hyperplastic lesions and tumours.
Materials and Methods: GLI1 expression was induced in female transgenic mice expressing GLI1 in the mammary gland. The mice were monitored for the occurrence of tumours. Palpable tumours and hyperplastic lesions developed in the mice with induced GLI1 expression. Normal and tumour tissue were analysed.
Results: We show that the cells of the basal cell layer of the large mammary ducts are Lgr5 positive. Lgr5 is also expressed in mammary gland tumours induced in conditionally transgenic mice expressing GLI1 in the mammary gland. Hyperplastic lesions and palpable mammary gland tumours also develop in nulliparous transgenic mice, after long term low level GLI1 expression. Both solid and acinar adenocarcinomas develop in GLI1 expressing nulliparous mice, even within the same mammary gland. The expression of the stem cell marker CD44 is increased in the mammary ducts as well as the tumours in the GLI1 expressing mice. The GLI1